

Forest Health Protection



Report 04-13

June 2004

PREPLANT SOIL TREATMENT EFFECTS ON PRODUCTION OF SEEDLINGS AT THE USDA FOREST SERVICE LUCKY PEAK NURSERY, BOISE, IDAHO

R. L. James, G. R. Knudsen, and M. J. Morra

ABSTRACT

Tests were conducted at the USDA Forest Service Lucky Peak Nursery to compare four pre-sowing treatments on production of bare root ponderosa pine seedlings. Treatments tested included standard fumigation with methyl bromide–chloropicrin (MBC), following with periodic cultivation, and two cultivars of *Brassica napus* incorporated into soil as green manure crops (“Dwarf Essex,” “Hybrid Mustard”). A biological control agent (*Trichoderma harzianum*) was also applied to some treatment areas. All treatments except MBC resulted in increased populations of *Fusarium* prior to sowing; the *Brassica* green manure crops greatly stimulated *Fusarium* populations. Seedling emergence, first-year seedling survival and height were less in all nonfumigation treatments. *Fusarium oxysporum* was the most frequently isolated pathogen from soil and diseased seedlings. Seedling height, diameter and root biomass on 2-0 seedlings were not significantly different among the treatments. Addition of *T. harzianum* had no effect on seedling production. Reduced seedling density, common in nonfumigation treatment areas, may result in larger seedlings.

INTRODUCTION

The USDA Forest Service Lucky Peak Nursery near Boise, Idaho has traditionally used preplant soil fumigation as a means of controlling soilborne pathogens, weeds, and improving bare root conifer seedling production. The fumigant of choice has been methyl bromide/chloropicrin (MBC), which is usually applied near the end or summer or early fall of the year prior to sowing when soil temperature and moisture conditions are most conducive for proper chemical soil penetration. Soil fumigation with MBC has resulted in sustained, predictable production

of high-quality seedlings at the nursery for many years (Marshall 1983).

Recently, methyl bromide has been targeted for phase out and elimination as a fumigant in the United States because of its propensity to degrade stratospheric ozone which affects penetration of ultraviolet light to the earth’s surface (Chapman 1992; Stone et al. 1995; World Meteorological Association 1995). The Montreal Protocol identified ozone-depleting chemicals and called for reduction and elimination of some of the more important ones (Evans and Greczy 1995; Shaheen 1996). Use of methyl bromide is currently

being reduced and its use is scheduled for elimination by January 2005, even though some exemptions may be granted. Therefore, several investigations have been conducted at the Lucky Peak Nursery to identify and test viable alternatives to preplant soil fumigation with methyl bromide. Previous tests have involved alternative chemical fumigants (dazomet) (James and Beall 1999) and bare fallowing fields for at least one growing season prior to sowing (James and Beall 2000). Neither of these treatments was deemed a viable alternative to methyl bromide.

Brassica spp. incorporated into soil as a green manure crop may provide alternatives to preplant soil fumigation with chemical pesticides. Metabolites from certain cultivars have shown toxicity to common soilborne plant pathogens (Mayton et al. 1996) and may exhibit disease-suppressive characteristics under agricultural conditions (Chung et al. 2002; Mayton et al. 1996; Ramirez-Villapudua and Munnecke 1988). Glucosinolates in *Brassica* tissues are converted to isothiocyanates upon decomposition (Mayton et al. 1996; Rosa and Rodrigues 1999); isothiocyanates, when produced in sufficient concentrations, may be toxic to certain soil pathogens. One way of supplementing possible disease suppressiveness of *Brassica* spp. is by incorporating biological control agents at the same time. *Trichoderma harzianum* has been successful in controlling soilborne pathogens in a number of agricultural settings (Knudsen and Bin 1990; Knudsen et al. 1991). This fungus competes with and produces toxic metabolites that may inhibit important soilborne pathogens (Knudsen and Bin 1990; Papavizas 1985).

An evaluation was conducted at the Lucky Peak Nursery to determine efficacy of selected *Brassica* cultivars as green manure crops, supplemented with application of *T. harzianum*, in controlling soilborne pathogens and production of high quality ponderosa pine seedlings.

MATERIALS AND METHODS

Tests were initiated during the summer of 2000. Each treatment was replicated three times; treatment blocks were located within three sections of Field 14 in a complete randomized block design (figure 1). Each treatment block was 50 ft. (16.5 m) in length and two seedling beds wide. The treatments were: standard methyl bromide/chloropicrin (MBC) fumigation in August 2000 at the rate of 350 lbs/acre (= 390 kg/ha), bare fallowing with periodic cultivation, and two *Brassica napus* green manure crops (cultivars "Dwarf Essex" and "Hybrid Mustard") that were sown in the late summer of 2000, grown over the winter, and incorporated into the soil 14 days before sowing in the spring of 2001. A formulation of *Trichoderma harzianum* (strain ThzID1) was added to half of the MBC, fallow, and *Brassica* plots just prior to sowing.

Soil populations of potential pathogens in the genus *Fusarium* and potential antagonists in the genus *Trichoderma* were assayed prior to soil treatments and again just prior to sowing. Samples were collected from within each replicate plot; three collections within each plot were mixed together to represent a single sample. Each collection consisted of a soil core taken to a depth of about 8 inches (20 cm). Soil was placed in plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard soil dilutions (Hildebrand and Dinkel 1988, James et al. 1990, 1996, Stone et al. 1995) were conducted to estimate populations of *Fusarium* and *Trichoderma* spp. Soil from each sample was initially sieved (2 mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5 g subsample was oven dried at about 100°C for at least 24 hrs. until sample weight stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For population assays, 0.05 g of field-moist soil was combined with 10 ml of



Figure 1. Plot layout in Field 14 to evaluate effects of preplant soil treatments on production of bare root ponderosa pine seedlings - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. Photo taken at the end of the first growing season.

0.3% water agar (WA) and thoroughly mixed. One milliliter of solution was placed on each of three plates of selective agar medium for *Fusarium* and closely related fungi (Komada 1975) and spread uniformly. *Trichoderma* propagules were also enumerated on Komada's medium which supports growth of this fungus unless amended with benomyl or lithium chloride. Plates were incubated for 7 days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations were expressed as number of colony-forming units (cfu) per g of oven-dried soil. Selected *Fusarium* isolates were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) for identification to species using the taxonomy of Nelson et al. (1983). Isolates of *Trichoderma* were not identified to species.

All plots were sown during early May 2001 with the same seedlot of ponderosa pine (*Pinus ponderosa* Laws.) using standard nursery procedures and covered with fine sand. After seedling emergence was deemed complete (mid-July), three sampling subplots (1.5 ft² – 0.31 m²) were installed within each replicate plot; these subplots were located approximately equidistant from each other and concentrated within the center of each replicate plot (figure 2). Seedling emergence and postemergence damping off were determined within each subplot in July. At the same time, 10 diseased seedlings were randomly collected from each replicate plot for pathogen analysis in the laboratory. Selected seedlings were washed thoroughly, root/stem tissues surface sterilized in a 0.525% aqueous solution of sodium hypochlorite, and dissected into pieces about 5 mm in length. Twenty-five randomly selected pieces from seedlings



Figure 2. Borders of subplots (white pot labels) used for determining seedling emergence, first-year survival, and level of disease within pre-plant treatment plots - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. Seedlings with postemergence disease are indicated by arrows.

within each replicate plot were placed on Komada's medium and isolated organisms were identified as described above for soil samples.

At the end of the first growing season (October), seedling density and disease were determined within each subplot. Heights of 10 seedlings within each subplot (if available) were measured at the same time. At the end of the first growing season, 100 diseased 1-0 pine seedlings were randomly collected from throughout the study area.

They were analyzed for pathogens by placing 5 root or stem pieces from each seedling on Komada's medium and identifying associated organisms as described above. Seedlings were washed and surface sterilized prior to isolations.

At the end of the second growing season (fall 2002), sample seedlings were carefully extracted from beds by hand for morphology measurements. Sample seedlings were located within inner seedling rows (to eliminate edge effects) and entire root systems were included with each seedling. Fifty "average" seedlings were collected from each replicate plot and transported to the

laboratory for measurement. Seedling height (from basal cotyledon scar to the tip of the terminal bud), diameter (just above the ground line) and root mass (oven-dry weight of all attached roots) were determined for each sample seedling. Seedling heights, diameters, and root masses were compared among the treatments with an analysis of variance. Significant differences ($P=0.05$) in these three morphology categories were located using the least significant difference (LSD) procedure.

RESULTS

Presowing fumigation with MBC greatly reduced, but did not eliminate soil populations of *Fusarium* (table 1). Levels of *Trichoderma* were also reduced, but not as drastically. The other nonfumigation treatments resulted in increased soil *Fusarium* populations, especially following incorporation of both *Brassica* green manure crops into soil. Soil *Fusarium* levels were well above estimated disease thresholds (> 1000 cfu/g) (Hildebrand and Dinkel 1988; James et al. 1990) in *Brassica* plots prior to sowing the conifer crop. Although *Fusarium* populations increased in the fallow treatments, presowing populations were slightly below disease threshold levels. Effects of the nonfumigation treatments on

Trichoderma populations were variable (table 1).

Seedling responses to preplant soil treatments were evaluated by measuring seedling emergence, first-year survival, disease and height growth, and height, diameter and root mass production at the end of the second growing season. Treatment effects on seedling emergence and postemergence damping-off are summarized in table 2. Reduced seedling emergence and higher damping-off occurred in nonfumigated plots. Biocontrol treatment improved seedling emergence for all treatments except soil fumigation; effects on damping off were variable. Isolations from seedlings with postemergence damping-off symptoms (figure

2) yielded high levels of *Fusarium oxysporum* Schlecht. (table 3). Three different morphotypes of this species were routinely isolated from diseased seedlings. Morphotypes were differentiated on their cultural variability, i.e., production of aerial mycelium, violet pigmentation, and sporodochia (pionnotal). All three morphotypes produced spores characteristic of *F. oxysporum*: chlamydospores, macroconidia, and microconidia borne in false heads on short, unbranched monophialides (Nelson et al. 1983). Two other *Fusarium* species (*F. sporotrichioides* Sherb. and *F. solani* (Mart.) Appel & Wollenw.) were isolated at very low levels (table 3). *Trichoderma* isolates were isolated infrequently from diseased seedlings.

Table 1. Effects of pre-plant treatments on soil populations of *Fusarium* and *Trichoderma* USDA Forest Service Lucky Peak Nursery, Boise, Idaho¹.

Treatment ²	Replication	Fusarium		Trichoderma	
		Pre-Treat	Pre-Sow	Pre-Treat	Pre-Sow
A1	1	168	0	4176	671
	2	452	34	2563	371
	3	486	0	3214	1212
	Average	340.7	11.2	3317.6	751.2
	% Change	- 96.7		- 77.4	
A2	1	349	1004	2775	1606
	2	954	706	3031	1847
	3	202	807	4297	1479
	Average	489.8	838.8	3321.9	1644.3
	% Change	+ 71.2		- 50.5	
A3	1	1015	3424	1863	2920
	2	469	19485	2853	1408
	3	1366	4232	2735	6827
	Average	945.1	9047.8	2527.9	3739.0
	% Change	+ 857.3		+ 47.9	
A4	1	242	1108	3131	2417
	2	442	9629	2141	3118
	3	188	607	3178	1651
	Average	290.5	3781.0	2816.7	2395.5
	% Change	+ 1201.5		- 14.9	

¹ Values in table are colony-forming units per gram of oven-dry soil. Percent change reflects changes from pre-treatment to pre-sowing populations.

² A1 = fumigation with methyl bromide/chloropicrin; A2 = bare fallow with periodic cultivation; A3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; A4 = cultivation and incorporation of Hybrid Mustard variety of *Brassica*.

Table 2. Effects of pre-plant soil treatments on emergence of ponderosa pine seedlings- USDA Forest Service Lucky Peak Nursery, Boise, Idaho¹.

Treatment Plot ²	With Biocontrol		Without Biocontrol	
	Emergence.	Disease	Emergence.	Disease
A-1-1	22.3	1.3	24.7	3.7
A-1-2	22.7	1.3	25.3	3.7
A-1-3	19.7	2.7	27.7	3.7
Average	21.5	1.8	25.9	3.7
A-2-1	21.3	5.7	14.3	3.3
A-2-2	22.7	4.0	19.3	2.7
A-2-3	11.7	4.3	19.0	2.0
Average	18.5	4.7	17.5	2.7
A-3-1	21.3	2.3	13.0	3.0
A-3-2	18.7	2.0	11.0	2.7
A-3-3	10.3	3.7	8.0	3.3
Average	16.8	2.7	10.7	3.0
A-4-1	18.0	3.3	12.3	2.0
A-4-2	16.0	3.0	9.3	2.7
A-4-3	15.3	3.7	4.3	5.0
Average	16.4	3.3	8.7	3.2

¹ Values in table are number of seedlings per 0.31m².

² A1 = fumigation with methyl bromide/chloropicrin; A2 = bare fallow with periodic cultivation; A3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; A4 = cultivation and incorporation of Hybrid Mustard variety of *Brassica*.

Table 3. *Fusarium* and *Trichoderma* isolated from diseased ponderosa pine seedlings at the end of seedling emergence - USDA Forest Service Lucky Peak Nursery, Boise, Idaho¹.

Treatment ²	Fusarium oxysporum³			Other <i>Fusarium</i> ⁴	Trichoderma
	Type 1	Type 2	Type 3		
A-1	88.0	32.0	9.3	0	9.3
A-2	78.6	60.0	16.0	1.3	6.7
A-3	88.0	70.7	24.0	0	14.7
A-4	69.3	65.3	13.3	1.3	8.0
All	81.0	57.0	15.6	0.7	9.7

¹ Values in table are percent of sampled root and stem pieces (75 sampled per treatment) colonized with the particular fungus.

² A1 = fumigation with methyl bromide/chloropicrin; A2 = bare fallow with periodic cultivation; A3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; A4 = cultivation and incorporation of Hybrid mustard variety of *Brassica*.

³ Types based on cultural characteristics (aerial hyphae, pigmentation, sporodochial production).

⁴ Includes *F. sporotrichioides* and *F. solani*.

Effects of preplant soil treatments on first-year seedling survival, height growth, and post-emergence disease are summarized in table 4. Seedling densities were less in all non-fumigation treatments, regardless of biocontrol agent application. Discernable seedling disease was low in all treatments. Seedlings were generally slightly taller within plots

fumigated with MBC, although differences among treatments were small.

Fusarium oxysporum was again the most commonly isolated pathogen from diseased 1-0 seedlings (table 5). All three morphotypes of this species were well represented in isolations. *Fusarium sporotrichioides* was also

isolated; *Trichoderma* spp. were isolated at low levels.

Preplant soil treatment effects on height, diameter, and root mass production of 2-0 seedlings are summarized in table 6. No significant differences were found in any of the measured morphology parameters among the four treatments. In addition, amendments with the biocontrol agent did not significantly affect seedling production. Similar-sized seedlings were produced from all treatments by the end of the second growing season.

DISCUSSION

Root diseases caused primarily by *Fusarium oxysporum* are important in limiting production of bare root pine seedlings at the Lucky Peak Nursery (James 2002; James and Beall 1999, 2000). Disease levels tend to vary among the different production fields and are also influenced by environmental factors, particularly moisture and ambient

temperatures. Most root disease occurs during the first growing season (James 2001, 2002); seedlings surviving the first year usually grow well the second season and reach desired sizes. However, seedling densities may be reduced by first-year root diseases and the required number of quality seedlings may not be obtained.

Preplant soil fumigation with MBC has been effective at the nursery for many years (Marshall 1983). Although expensive, fumigation has generally resulted in denser seedbeds and larger, healthier seedlings. Because of the impending loss of methyl bromide as a fumigant, several trials have been conducted at the nursery to identify viable alternatives. The fumigant dazomet (Basamid®) was not as effective at Lucky Peak as other nurseries (James and Beall (1999), primarily because heavy soils with

Table 4. Effects of pre-plant soil treatments on first-year seedling survival, disease, and height growth - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Treatment Plot ¹	With Biocontrol			Without Biocontrol		
	Live ²	Dead ²	Height ³	Live ²	Dead ²	Height ³
A-1-1	20.7	1.3	6.02	23.0	1.3	7.25
A-1-2	22.0	1.3	7.65	21.3	1.3	8.25
A-1-3	24.3	0.7	8.17	19.7	1.7	9.70
Average	22.3	1.1	7.28	21.3	1.4	8.40
A-2-1	20.7	0.7	7.05	13.0	0.7	8.39
A-2-2	17.7	2.0	7.22	20.0	0.7	8.67
A-2-3	10.0	2.0	7.30	16.3	1.0	7.78
Average	16.1	1.5	7.19	16.4	0.8	8.28
A-3-1	18.7	1.3	6.70	13.3	0.7	7.55
A-3-2	10.7	0.3	7.02	16.3	2.0	7.12
A-3-3	6.7	1.0	7.27	8.3	1.7	6.76
Average	12.0	0.9	6.96	12.7	1.4	7.16
A-4-1	15.3	0.3	6.81	13.0	0.3	7.40
A-4-2	14.0	2.3	7.68	8.0	0.7	7.85
A-4-3	10.3	0.7	7.89	6.3	1.3	9.32
Average	13.2	1.1	7.44	9.1	0.8	8.05
All	15.9	1.2	7.22	14.9	1.1	7.98

¹ A1 = fumigation with methyl bromide/chloropicrin; A2 = bare fallow with periodic cultivation; A3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; A4 = cultivation and incorporation of Hybrid Mustard variety of *Brassica*

² Number of seedlings per 0.31m².

³ Average height of 10 seedlings - if available (cm).

Table 5. *Fusarium* and *Trichoderma* isolated from diseased ponderosa pine seedlings at the end of the first growing season - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.¹

Fungus	Infection ¹	Colonization ²
<i>Fusarium oxysporum</i> ³	82	68.2
Type 1		
Type 2	82	60.4
Type 3	62	26.0
All Isolates	100	100
<i>Fusarium sporotrichioides</i>	2	0.8
<i>Trichoderma</i>	20	5.0

¹ Percent of 100 seedlings sampled infected with the particular fungus.

² Percent of stem and root pieces sampled (5 per seedling) colonized with the particular fungus.

³ Types based on cultural characteristics (aerial hyphae, pigmentation, sporodochial production).

Table 6. Effects of pre-plant soil treatment on 2-0 seedling density, height, diameter, and root biomass - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Treatment ¹	Average Height (cm)	Average Diameter (mm)	Average Root Mass (g)
A1 + Biocontrol	25.8	6.2	2.54
A1 – Biocontrol	22.7	5.4	1.94
A2 + Biocontrol	23.1	6.1	2.78
A2 – Biocontrol	21.7	5.9	3.07
A3 + Biocontrol	21.7	6.4	3.21
A3 – Biocontrol	22.2	6.2	2.94
A4 + Biocontrol	22.1	6.6	3.50
A4 – Biocontrol	22.5	6.1	2.99
All "A1"	24.3 A ²	5.8 A ²	2.24 A ²
All "A2"	22.4 A	6.0 A	2.93 A
All "A3"	22.0 A	6.3 A	3.08 A
All "A4"	22.3 A	6.4 A	3.25 A
All + Biocontrol	23.2 A ³	6.3 A ³	3.01 A ³
All – Biocontrol	22.3 A	5.9 A	2.74 A

¹ A1 = fumigation with methyl bromide/chloropicrin; A2 = bare fallow with periodic cultivation; A3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; A4 = cultivation and incorporation of Hybrid Mustard variety of *Brassica*. Biocontrol treatments were addition of *Trichoderma harzianum* strain ThzID1.

² For comparisons among the four treatments, means followed by the same letter are not statistically different (P=0.05) using the least significant difference (LSD) procedure.

³ For comparisons between treatments with and without biocontrol amendments, means followed by the same letter are not statistically different (P=0.05) using the least significant difference (LSD) procedure.

high clay content restricted distribution of the fumigant throughout the soil profile (Munnecke and Van Gundy 1979). Likewise, fallowing fields with periodic cultivation was not very effective in reducing soil pathogen inoculum or improving seedling production, particularly in

fields with heavier soils and poorer water drainage (James and Beall 2000).

Our results indicated that incorporating *Brassica* green manure crops into soil initially caused extensive increases in soil *Fusarium* populations, including *F. oxysporum*. This

population increase adversely affected seedling emergence and growth during the first growing season. Higher levels of pre-emergence disease probably resulted from increased *Fusarium* levels; postemergence disease may not have been as severe. However, during the second growing season, seedlings were much less affected. Seedlings surviving to the end of the second growing season grew well and were generally healthy regardless of the preplant treatment. By the time of lifting, seedlings produced in all areas were similar. Therefore, the most important benefit of soil fumigation was first-year seedling establishment and performance. Reductions in seedling density may occur because of higher first-year root disease. However, root diseases did not greatly affect seedlings during the second growing season. If sufficient area is available for bare root seedling production at the nursery, reduced seedling densities may not be of concern. However, high first-year disease means that sowing densities must remain high to achieve desired number of seedlings.

Biological control with one strain of *Trichoderma harzianum* has thus far not effectively controlled soilborne diseases in tested forest nurseries (James 2000; James et al. 2004). We found little effect of this biocontrol agent in our tests, other than limited improvement in seedling emergence in plots treated with *Brassica* green manure crops. There may have been some amelioration of resident *Fusarium* populations by the biocontrol agent, but this was difficult to measure.

Tests of *Brassica* green manure crops to reduce soilborne diseases in forest nurseries have so far been disappointing. Although such treatments were effective in some agricultural systems (Angus et al. 1994; Chung et al. 2002), similar results have not been forthcoming in forest nurseries (Hansen et al. 1990; Hamm and Hansen 1990; James et al. 2004). It is likely that the level of glucosinolates produced by tested *Brassica* cultivars following soil incorporation is insufficient to offset the proliferation of pathogen populations that occur because of

adding large amounts of organic matter (Hansen et al. 1990). This could be caused by inefficient release of isothiocyanates from the tissues, a key step that requires extensive tissue maceration and ample water (Morra and Kirkegaard 2002). Alternatively, glucosinolates present within *B. napus* tissues may not produce products that are biologically active against *F. oxysporum*. The latter possibility is plausible, given the fact that *B. napus* does not contain glucosinolates that release 2-propenyl isothiocyanate (Gardiner et al. 1999), an isothiocyanate that was shown to inhibit mycelial growth and completely suppress conidial and chlamydospore germination (Smolinska et al. 2003).

Fusarium spp. are excellent saprophytes on a wide range of organic matter. Their populations respond directly to soil additions of organic matter and may stabilize when food sources become scarcer. *Fusarium oxysporum* responds to added organic matter like other *Fusarium* spp. This fungal species makes up the vast majority of the *Fusarium* population at the Lucky Peak Nursery (James and Beall 1999, 2000) and is almost exclusively the species isolated from diseased as well as healthy seedling root systems (James 2000, 2001, 2002). Not all isolates of *F. oxysporum* are pathogens (Gordon and Martyn 1997; Gordon and Okamoto 1992). However, pathogenic and nonpathogenic isolates appear morphologically similar and cannot easily be differentiated. Controlled pathogenicity tests have been successful in locating pathogenic strains (James et al. 2000), but more promising techniques include molecular genetic analyses (Assighetse et al. 1994; Gordon and Martyn 1997; Kim et al. 1993; Kistler 1997). If molecular probes can be developed to locate pathogenic strains of *F. oxysporum* from forest nurseries, growers will be in a much better position to predict disease severity from information about resident populations of this fungus.

The Lucky Peak Nursery has a long history of producing high-quality bare root forest seedlings, particularly pine species. This has been possible by routine preplant soil fumigation with MBC. The future without

methyl bromide may result in greater problems with bare root seedling production, primarily because of expected higher levels of root disease. This may partly be overcome by conversion of production to containers, which is being actively pursued at the nursery. For example, a new greenhouse facility was recently installed which will be able to produce thousands of container seedlings annually. This will likely reduce the need for but will not eliminate bare root production in the future. Therefore, continued efforts to identify viable alternatives to methyl bromide fumigation at the Lucky Peak Nursery will be necessary.

LITERATURE CITED

- Angus, J.F., P.A. Gardner, J.A. Kirkegaard and J.M. Desmarchelier. 1994. Biofumigation: isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. *Plant and Soil* 162:107-112.
- Assigbetse, K.B., D. Fernandez, M.P. Dubois and J.-P. Geiger. 1994. Differentiation of *Fusarium oxysporum* f.sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPDs) analysis. *Phytopathology* 84:622-626.
- Chapman, W. 1992. Alternative treatments to methyl bromide. *In*: Conference Proceedings: Southern Forest Nursery Association 1992:96-103.
- Chung, W.C., J.W. Huang, H.C. Huang and J.F. Jen. 2002. Effect of ground *Brassica* seed meal on control of *Rhizoctonia* damping-off of cabbage. *Canadian Journal of Plant Pathology* 24:211-218.
- Evans, G.R. and L.M. Greczy. 1995. Methyl bromide: the cure-all of the horticulture industry will be banned by 2001. When this happens, what, if anything, will take its place. *American Nurseryman* 182(7):95-105.
- Fisher, N.L., L.W. Burgess, T.A. Toussoun and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72:151-153.
- Gardiner, J.B., M.J. Morra, C.V. Eberlein, P.D. Brown and V. Borek. 1999. Allelochemicals released in soil following incorporation of rapeseed (*Brassica napus*) green manures. *Journal of Agricultural and Food Chemistry* 47:3837-3842.
- Gordon, T.R. and R.D. Martyn. 1997. The evolutionary biology of *Fusarium oxysporum*. *Annual Review of Phytopathology* 35:111-128.
- Gordon, T.R. and D. Okamoto. 1992. Population structure and the relationship between pathogenic and non-pathogenic strains of *Fusarium oxysporum*. *Phytopathology* 82:73-77.
- Hansen, E.M., D.D. Myrold and P.B. Hamm. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. *Phytopathology* 80:698-704.
- Hamm, P.B. and E.M. Hansen. 1990. Soil fumigation, cover cropping, and organic soil amendments: their effect on soil-borne pathogens and the target seedlings. *In*: Rose, R., S.J. Campbell and T.D. Landis (eds.). *Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations*. USDA Forest Service, Rocky Mountain Experiment Station. General Technical Report RM-200. pp. 174-180.
- Hildebrand, D.M. and G.B. Dinkel. 1988. Evaluation of methyl bromide, Basamid granular, and solar heating for pre-plant pest control for fall-sown eastern redcedar at Bessey Nursery. USDA Forest Service, Rocky Mountain Region, Timber, Forest Pest, and Cooperative Forestry Management. Technical Report R2-41. 13p.
- James, R.L. 2000. Effects of topical application of the biological control agent Biotrek® on production of bareroot Douglas-fir and western white pine seedlings – USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern

- Region, Forest Health Protection. Report 00-5. 8p.
- James, R.L. 2001. Effects of pre-sowing soil treatments on root colonization of 1-0 ponderosa and lodgepole pine seedlings by potentially-pathogenic fungi - USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 01-9. 9p.
- James, R.L. 2002. Effects of preplant soil treatments on *Fusarium* and *Trichoderma* populations and fungal root colonization of 1-0 nondiseased ponderosa pine seedlings - USDA Forest Service Lucky Peak Nursery – Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 02-3. 9p.
- James, R.L. and K. Beall. 1999. An evaluation of the effects of dazomet on soil-borne diseases and conifer seedling production- USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 99-9. 15p.
- James, R.L. and K. Beall. 2000. Effects of fallowing on *Fusarium*-associated root diseases and production of bare root ponderosa pine seedlings at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 00-3. 13p.
- James, R.L., G.R. Knudsen and M.J. Morra. 2004. Effects of pre-plant soil treatments on production of Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 04-10. 14p.
- James, R.L., S. Metzger and C.J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-11. 18p.
- James, R.L., D.S. Page-Dumroese, S.K. Kimball and S. Omi. 1996. Effects of *Brassica* cover crop, organic amendment, fallowing, and soil fumigation on production of bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-6. 10p.
- James, R.L., R. Perez, R.K. Dumroese and D.L. Wenny. 2000. Virulence of *Fusarium oxysporum* on Douglas-fir germinants: comparison of isolates from nursery soil and roots of healthy and diseased seedlings. In: Lilja, A. and J.R. Sutherland (eds.). Proceedings of the 4th Meeting of IUFRO Working Party 7.03.04 – Diseases and Insects in Forest Nurseries. Finnish Forest Research Institute, Research Papers 781. pp. 49-64.
- Kim, D.H., R.D. Martyn and C.W. Magill. 1993. Mitochondrial DNA (mtDNA) relatedness among formae speciales of *Fusarium oxysporum* in the Cucurbitae. *Phytopathology* 83:91-97.
- Kistler, H.C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathology* 87:474-479.
- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research (Japan)* 8:114-125.
- Knudsen, G.R. and L. Bin. 1990. Effects of temperature, soil moisture, and wheat bran on growth of *Trichoderma harzianum* from alginate pellets. *Phytopathology* 80:724-727.
- Knudsen, G.R., D.J. Eschew, L.M. Dandurand and Z.G. Wang. 1991. Method to enhance growth and sporulation of pelletized biocontrol fungi. *Applied and Environmental Microbiology* 57:2864-2867.
- Marshall, J.P. 1983. Effectiveness of methyl bromide/chloropicrin fumigation in reducing *Fusarium* populations in two major soil types

- at the USDA Forest Service Lucky Peak Nursery. USDA Forest Service, Intermountain Region, Forest Pest Management. Report 83-6. 9p.
- Mayton, H.S., C. Oliviker, S.F. Vaughn and R. Loria. 1996. Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* 86:267-271.
- Morra, M.J. and J.A. Kirkegaard. 2002. Isothiocyanate release from soil-incorporated *Brassica* tissues. *Soil Biology & Biochemistry* 34:1683-1690.
- Munnecke, D.E. and S.D. Van Gundy 1979. Movement of fumigants in soil, dosage responses, and differential effects. *Annual Review of Phytopathology* 17:405-429.
- Nelson, P.E. T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park. 193p.
- Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. *Annual Review of Phytopathology* 23:23-54.
- Ramirez-Villapudua, J. and D.E. Munnecke. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f.sp. *conglutinans* and other organisms. *Phytopathology* 78:289-295.
- Rosa, E.A.S. and P.M.F. Rodrigues. 1999. Towards a more sustainable agriculture system: The effect of glucosinolates on the control of soil-borne diseases. *Journal of Horticultural Science & Biotechnology* 74:667-674.
- Shaheen, L. 1996. Potential loss of methyl bromide to prompt changes in Clean Air Act. *Pest Control* 64(5):68,74
- Smolinska, U., M.J. Morra, G.R. Knudsen and R. L. James. 2003. Isothiocyanates produced by Brassicaceae species as inhibitors of *Fusarium oxysporum*. *Plant Disease* 87:407-412.
- Stone, J.K., D.M. Hildebrand, R.L. James, S.M. Frankel and D.S. Germandt. 1995. Alternatives to methyl bromide for control of soil-borne diseases in bare root forest nurseries. *In: Proceedings: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions*. November 6-8, 1995, San Diego, CA. Methyl Bromide Alternatives Outreach, US Environmental Protection Agency and US Department of Agriculture. pp. 77-1 – 77-4.
- World Meteorological Association. 1995. Scientific assessment of ozone depletion: 1994 executive summary. *Global Ozone Research and Monitoring Project Report No. 37*. Global Ozone Observing System, Geneva, Switzerland. 36p.

R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection. Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83814; email rjames@fs.fed.us. G.R. Knudsen and M.J. Morra are with the Soil & Land Resources Division, University of Idaho, Moscow 83844-2339; email: (G.R. Knudsen): microbes@moscow.com; (M.J. Morra): mmorra@uidaho.edu.